Copy f r the Elected Office (EO/US) 50 CO PCT/US98/26469

PATENT COOPERATION TREATY

09/555987

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

GANDY, Kenneth, A.
Woodard, Emhardt, Naughton,
Moriarty & McNett
Bank One Center/Tower
Suite 3700
111 Monument Circle
Indianapolis, IN 46204
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
16 June 2000 (16.06.00)	
Applicant's or agent's file reference PUR997024344	
International application No. PCT/US98/26469	**International filing date** (day/month/year) 11 December 1998 (11.12.98)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
PECK, Louise, W. 3200 W. 450 North West Lafayette, IN 47906 United States of America	US	US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
PECK, Louise, W. 430 E. Lewis Street Moscow, ID 83843 United States of America	US	US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Simin Baharlou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 003356132

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 August 1999 (23.08.99)	
International application No. PCT/US98/26469	**Applicant's or agent's file reference** PUR997024344
International filing date (day/month/year) 11 December 1998 (11.12.98)	**Priority date** (day/month/year) 12 December 1997 (12.12.97)
Applicant VANDEN HEUVEL, John, P. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

12 July 1999 (12.07.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

R. Forax

Telephone No.: (41-22) 338.83.38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* PUR997024344

Box No. I TITLE OF INVENTION

METHODS AND COMPOSITIONS FOR TREATING DIABETES

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PURDUE RESEARCH FOUNDATION
Office of Technology Transfer
1063 Hovde Hall
West Lafayette, Indiana 47907
United States of America

☐ This person is also inventor.

Telephone No. 765-494-2610

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

THE PENN STATE RESEARCH FOUNDATION
304 Old Main
University Park, Pennsylvania 16802
United States of America

This person is:

☒ applicant only

☐ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

GANDY, Kenneth A.
WOODARD, EMHARDT, NAUGHTON, MORIARTY & MCNETT
Bank One Center/Tower, Suite 3700
111 Monument Circle
Indianapolis, Indiana 46204 US

SEE CONTINUATION TO BOX NO. IV ON SHEET NO. 4

Telephone No. 317-634-3456

Facsimile No. 317-637-7561

Teleprinter No. 810-341-3283

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

See Notes to the request form

Continuation f Box N . III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant 's State (that is, country) of residence if no State of residence is indicated below.)*

VANDEN HEUVEL, John P.
101 James Hill Road
Port Matilda, Pennsylvania 16807
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [XX] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant 's State (that is, country) of residence if no State of residence is indicated below.)*

BELURY, Martha A.
181 Ivy Hill Drive
West Lafayette, Indiana 47906
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant 's State (that is, country) of residence if no State of residence is indicated below.)*

PECK, Louise W.
3200 W. 450 North
West Lafayette, Indiana 47906
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant 's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* f nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/ r (further) invent rs are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes: at least one must be marked)*:

Regional Patent

- [X] AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* GW – Guinea-Bissau

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] AL Albania
- [X] AM Armenia
- [X] AT Austria
- [X] AU Australia
- [X] AZ Azerbaijan
- [X] BA Bosnia and Herzegovina
- [X] BB Barbados
- [X] BG Bulgaria
- [X] BR Brazil
- [X] BY Belarus
- [X] CA Canada
- [X] CH and LI Switzerland and Liechtenstein
- [X] CN China
- [X] CU Cuba
- [X] CZ Czech Republic
- [X] DE Germany
- [X] DK Denmark
- [X] EE Estonia
- [X] ES Spain
- [X] FI Finland
- [X] GB United Kingdom
- [X] GE Georgia
- [X] GH Ghana
- [X] GM Gambia
- [X] GW Guinea-Bissau
- [X] HR Croatia
- [X] HU Hungary
- [X] ID Indonesia
- [X] IL Israel
- [X] IS Iceland
- [X] JP Japan
- [X] KE Kenya
- [X] KG Kyrgyzstan
- [X] KP Democratic People's Republic of Korea
- [X] KR Republic of Korea
- [X] KZ Kazakhstan
- [X] LC Saint Lucia
- [X] LK Sri Lanka
- [X] LR Liberia
- [X] LS Lesotho
- [X] LT Lithuania
- [X] LU Luxembourg
- [X] LV Latvia
- [X] MD Republic of Moldova
- [X] MG Madagascar
- [X] MK The former Yugoslav Republic of Macedonia
- [X] MN Mongolia
- [X] MW Malawi
- [X] MX Mexico
- [X] NO Norway
- [X] NZ New Zealand
- [X] PL Poland
- [X] PT Portugal
- [X] RO Romania
- [X] RU Russian Federation
- [X] SD Sudan
- [X] SE Sweden
- [X] SG Singapore
- [X] SI Slovenia
- [X] SK Slovakia
- [X] SL Sierra Leone
- [X] TJ Tajikistan
- [X] TM Turkmenistan
- [X] TR Turkey
- [X] TT Trinidad and Tobago
- [X] UA Ukraine
- [X] UG Uganda
- [X] US United States of America
- [X] UZ Uzbekistan
- [X] VN Viet Nam
- [X] YU Yugoslavia
- [X] ZW Zimbabwe

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- [X] GD – Grenada
- [X] IN – India

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

(vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;

(vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

Continuation to Box No. IV Agent

WOODARD, Harold R.; EMHARDT, C. David; NAUGHTON, Joseph A., Jr.; MORIARTY, John V.; McNETT, John C.; HENRY, Thomas Q.; DURLACHER, James M.; REEVES, Charles R.; WAGNER, Vincent O.; ZLATOS, Steve; BEREVESKOS, Spiro; BAHRET, William F.; BROWNING, Clifford W.; FRISK, R. Randall; LUEDERS, Daniel J.; GANDY, Kenneth A.; THOMAS, Timothy N.; SISSELMAN, Kerry P.; JONES, Kurt N.; ALLIE, John H.; BANTA, Holiday W.; COLE, Troy J.; PAYNTER, L. Scott; LOWES, J. Andrew; MEYER, Charles J.; HARRIS, Darrin Wesley; SCHANTZ, Matthew R.; COY, Gregory B.; HIDAY, Lisa A.; DANILUCK, John V.; BROWN, Christopher A.; USHER, A.J., IV; MYERS, James B., Jr.; ROWE, James L. and STEVENS, Scott J., all of Woodard, Emhardt, Naughton, Moriarty & McNett, Bank One Center/Tower, Suite 3700, 111 Monument Circle, Indianapolis, Indiana 46204 United States of America

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) (12.12.97) 12 December 1997	60/069,567	US		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1)

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*: ISA / US

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date (day/month/year)	Number	Country (or regional Office)
12 December 1997	60/069,567	US

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request	:	5
description (excluding sequence listing part)	:	28
claims	:	4
abstract	:	1
drawings	:	10
sequence listing part of description	:	—
Total number of sheets	:	48

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Transmittal Letter (dup)

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Applicant(s):

PURDUE RESEARCH FOUNDATION
THE PENN STATE RESEARCH FOUNDATION
(VANDEN HEUVEL, John P.)
(BELURY, Martha A.)
(PECK, Louise W.)

Agent: Kenneth A. Gandy

(Kenneth A. GANDY)

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: PUR997024344

Applicant: PURDUE RESEARCH FOUNDATION, et al.

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . 240 T

2. SEARCH FEE . . . . . 700 S

International search to be carried out by US

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 48 sheets.

first 30 sheets 455 b1

18 (remaining sheets) x 10 (additional amount) = 180 b2

Add amounts entered at b1 and b2 and enter total at B . . . . 635 B

Designation Fees

The international application contains 78 designations.

11 (number of designation fees payable *(maximum 11)*) x 105 (amount of designation fee) = MAX 1155 D

Add amounts entered at B and D and enter total at I . . . . . 1790 I

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . 15 P

5. TOTAL FEES PAYABLE . . . . . $ 2745.00 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

- ☒ authorization to charge deposit account (see below)
- ☒ cheque
- ☐ postal money order
- ☐ bank draft
- ☐ cash
- ☐ revenue stamps
- ☐ coupons
- ☐ other *(specify)*:

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ US

- ☐ is hereby authorized to charge the total fees indicated above to my deposit account.
- ☒ is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.
- ☐ is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

23-3030 — Deposit Account No.

11 December 1998 — Date *(day/month/year)*

Signature Kenneth A. GANDY, #33,386

PCT INTERNATIONAL APPLICATION TRANSMITTAL LETTER | DATE 1 December 1998

REGARDING THE INTERNATIONAL APPLICATION OF PURDUE RESEARCH FOUNDATION, et al. | DOCKET OR REFERENCE NUMBER PUR997024344

ENTITLED METHODS AND COMPOSITIONS FOR TREATING DIABETES 09/555987

Certification under 37 CFR 1.10 (if applicable)

EM577549565US — "Express Mail" mailing number

11 December 1998 — Date of Deposit

I hereby certify that this application is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

LINDA C. SHELBY (Typed or printed name of person mailing application)

Linda C. Shelby (Signature of person mailing application)

To the United States Receiving Office (RO/US):

Accompanying this transmittal letter is the above-identified International application, including a completed Request form (PCT/RO/101). Please process the application according to the provisions of the Patent Cooperation Treaty.

The following requests are made of the RO/US:

1. [X] PREPARATION AND TRANSMITTAL OF CERTIFIED COPY OF PRIORITY DOCUMENTS—Please prepare and transmit to the International Bureau a certified copy of the United States origin priority documents identified in Box VI of the Request form (37 CFR 1.451).

   To cover the cost of copy preparation and certification (37 CFR 1.19(a)(3) and (b)(1)),

   [X] a (check) (money order) in the amount of $ 15.00 included in Fee is attached to this transmittal letter.

   [ ] the RO/US is hereby authorized to charge the following deposit account no.: _____.

2. [X] CHOICE OF INTERNATIONAL SEARCHING AUTHORITY—It is requested that the International Search be performed by the following International Searching Authority:

   [X] United States Patent and Trademark Office (ISA/US)

   [ ] European Patent Office (ISA/EP)

   The appropriate Search fee for the above-named Authority is indicated on the Fee Calculation Sheet (PCT/RO/101 Annex).

3. [X] SUPPLEMENTAL SEARCH FEES (ONLY WHEN ISA/US CONDUCTS THE INTERNATIONAL SEARCH.)—Please charge any Supplemental Search fees that may be required by the United States International Searching Authority (ISA/US) to deposit account no.: # 23-3030.

   I understand that this authorization is subject to my oral confirmation thereof in each instance and that it in no way limits my right to submit a protest against payment of the Supplemental Search fees, but is merely an administrative aid to assure that the ISA/US may timely complete the Search Report.

NOTE: SUPPLEMENTAL SEARCH FEES FOR ISA/EP ARE PAYABLE DIRECTLY TO THE EUROPEAN PATENT OFFICE

4. [X] DISCLOSURE INFORMATION—In order to assist in screening the accompanying International application for purposes of determining whether a license for foreign transmittal should and could be granted and for other purposes, the following information is supplied:

   A. [ ] There is no prior filed application relating to this invention.

   B. [X] There is a prior application, serial number 60/069,567 filed on 12 December 1997 (12.12.97) which contains subject matter that is

      1. [X] substantially identical to that of the accompanying International application.

      2. [ ] less than that of the accompanying International application. The additional subject matter of the International application appears on pages(s) and line(s) _____.

      3. [ ] more than that of the accompanying International application.

   C. [ ] Disclosure information cannot be covered by the language of Points 4A or 4B above due to the involvement of several prior applications or for other reasons. A separate sheet on which the disclosure information is explained is attached to this transmittal letter.

5. [X] REQUEST FOR FOREIGN TRANSMITTAL LICENSE—According to the provisions of 35 U.S.C. 184 and 37 CFR 5.11, a license to transmit the accompanying International application to foreign agencies or international authorities is hereby requested.

SIGNER IS THE
[ ] APPLICANT
[ ] COMMON REPRESENTATIVE
[X] (ATTORNEY) (AGENT) REG NO #33,386

NAME OF SIGNER (typed): Kenneth A. Gandy

SIGNATURE: Kenneth A. Gandy

M·H

17

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PUR997024344	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US98/26469	International filing date *(day/month/year)* 11 DECEMBER 1998	Priority date *(day/month/year)* 12 DECEMBER 1997
International Patent Classification (IPC) or national classification and IPC IPC(6): A61K 31/22, 31/225 and US Cl.: 514/546, 547		
Applicant PURDUE RESEARCH FOUNDATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12 JULY 1999	13 JANUARY 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer THEODORE J. CRIARES
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

Form PCT/IPEA/409 (cover sheet) (January 1994)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/26469

I. Basis of the report

1. This report has been drawn on the basis of (*Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments*):

[X] the international application as originally filed.

[X] the description, pages 1-29 , as originally filed.
pages NONE , filed with the demand.
pages NONE , filed with the letter of _____.
pages _____ , filed with the letter of _____.

[X] the claims, Nos. 1-21 , as originally filed.
Nos. NONE , as amended under Article 19.
Nos. NONE , filed with the demand.
Nos. NONE , filed with the letter of _____.
Nos. _____ , filed with the letter of _____.

[X] the drawings, sheets/~~fig~~ 1-10 , as originally filed.
sheets/~~fig~~ NONE , filed with the demand.
sheets/~~fig~~ NONE , filed with the letter of _____.
sheets/~~fig~~ _____ , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[X] the description, pages NONE.

[X] the claims, Nos. NONE.

[X] the drawings, sheets/~~fig~~ NONE.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/26469

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims	5-21	YES
	Claims	1-4	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-21	NO
Industrial Applicability (IA)	Claims	1-21	YES
	Claims	NONE	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-4 lack novelty under PCT Article 33(2) as being anticipated by Mendy or Bistrian et al.

Mendy at column 1, line to column 6, line 58 teach the use of conjugated linoleic acid to treat diabetes.

Bistrian et al. teach at column 3, line 64 to column 4, line 40 teach the use of linoleic acids to treat diabetes.

It is deemed that these teachings establish claims 1-4 lack novelty under PCT Article 33(2).

Claims 1-21 lack an inventive step under PCT Article 33(3) as being obvious over Mendy or Bistrian et al. As stated above each of the references cited teach the use of linoleic acids useful in the treatment of diabetes. The skilled artisan would have been motivated to use other linoleic acids to treat diabetes and expect success in view of these teachings. Therefore, claims 1-21 lack an inventive step under PCT Article 33(3).

Claims 1-21 have industrial applicability as defined by PCT Article 33(4) since these are useful in the pharmaceutical industry.

Applicant's Response to Written Opinion filed 15 December 1999 stating:
"Applicant does notwish to make any amendments
at this time"
has been entered in the file.

---------- NEW CITATIONS ----------

NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: KENNETH A. GANDY
WOODARD, EMHARDT, NAUGHTON,
MORIARTY & MCNETT
BANK ONE CENTER/TOWER
SUITE 3700, 111 MONUMENT CIRCLE
INDIANAPOLIS, IN 46204

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	27 JAN 2000

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
PUR997024344	

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US98/26469	11 DECEMBER 1998	12 DECEMBER 1997

Applicant
PURDUE RESEARCH FOUNDATION

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	THEODORE J. CRIARES
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

Form PCT/IPEA/416 (July 1992)★

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PUR997024344	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US98/26469	International filing date *(day/month/year)* 11 DECEMBER 1998	Priority date *(day/month/year)* 12 DECEMBER 1997
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Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

Form PCT/IPEA/409 (cover sheet) (January 1994)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/26469

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pages NONE , filed with the demand.
pages NONE , filed with the letter of _____.
pages _____ , filed with the letter of _____.

[X] the claims, Nos. 1-21 , as originally filed.
Nos. NONE , as amended under Article 19.
Nos. NONE , filed with the demand.
Nos. NONE , filed with the letter of _____.
Nos. _____ , filed with the letter of _____.

[X] the drawings, sheets/~~fig~~ 1-10 , as originally filed.
sheets/~~fig~~ NONE , filed with the demand.
sheets/~~fig~~ NONE , filed with the letter of _____.
sheets/~~fig~~ _____ , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[X] the description, pages NONE.

[X] the claims, Nos. NONE.

[X] the drawings, sheets/~~fig~~ NONE.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/26469

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

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	Claims	1-4	NO
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	Claims	1-21	NO
Industrial Applicability (IA)	Claims	1-21	YES
	Claims	NONE	NO

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Claims 1-21 have industrial applicability as defined by PCT Article 33(4) since these are useful in the pharmaceutical industry.

Applicant's Response to Written Opinion filed 15 December 1999 stating:
"Applicant does notwish to make any amendments
at this time"
has been entered in the file.

---------- NEW CITATIONS ----------
NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/26469

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 31/22, 31/225
US CL :514/546, 547
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/546, 547

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: linoleic and diabetes

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,407,821 A (MENDY) 04 October 1983, column 1, line 6 to column 6, line 58.	1-21
Y	US 4,871,768 A (BISTRIAN et al.) 03 October 1989, column Column 3, line 64 to column 4, line 40.	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"&" document member of the same patent family	

Date of the actual completion of the international search 23 MARCH 1999	Date of mailing of the international search report 12 APR 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer THEODORE J. CRIARES Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/22, 31/225

A1

(11) International Publication Number: **WO 99/29317**

(43) International Publication Date: 17 June 1999 (17.06.99)

(21) International Application Number: PCT/US98/26469

(22) International Filing Date: 11 December 1998 (11.12.98)

(30) Priority Data:
60/069,567 12 December 1997 (12.12.97) US

(71) Applicants *(for all designated States except US)*: PURDUE RESEARCH FOUNDATION [US/US]; Office of Technology Transfer, 1063 Hovde Hall, West Lafayette, IN 47907 (US). THE PENN STATE RESEARCH FOUNDATION [US/US]; 304 Old Main, University Park, PA 16802 (US).

(72) Inventors; and
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(74) Agents: GANDY, Kenneth, A. et al.; Woodard, Emhardt, Naughton, Moriarty & McNett, Bank One Center/Tower, Suite 3700, 111 Monument Circle, Indianapolis, IN 46204 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS AND COMPOSITIONS FOR TREATING DIABETES

(57) Abstract

Methods of treating diabetes in an animal and food compositions useful for treating diabetes are described. In one aspect of the invention, the method includes treating the animal with a therapeutically effective amount of CLA including 9,11–octadecadienoic acid and 10,12–octadecadienoic acid, isomers thereof, esters thereof, salts thereof or mixtures thereof. In another aspect of the invention, a food composition comprising a food product having a therapeutically effective amount of a purified CLA isomer, including cis,cis–9,11–octadecadienoic acid, trans,cis–10,12–octadecadienoic acid or a mixture of purified cis,trans–9,11–octadecadienoic acid and trans,cis–9,11–octadecadienoic acid is described.

FOR THE PURPOSES OF INFORMATION ONLY

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09/555987
PCT/US98/26469
533 Rec'd PCT/PTO 07 JUN 2000

METHODS AND COMPOSITIONS FOR TREATING DIABETES

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Provisional Patent Application Serial Number 60/069,567, filed on December 12, 1997, which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

The present invention relates generally to methods of treating diabetes. Specifically, the invention relates to methods of treating diabetes in an animal by administering a therapeutically effective amount of conjugated linoleic acid (CLA). The invention further relates to food compositions including a food product having a therapeutically effective amount of a purified isomer of CLA, such as purified cis,cis-9,11-octadecadienoic acid, purified trans,cis-10,12-octadecadienoic acid or a mixture of purified cis,trans-9,11-octadecadienoic acid and trans,cis-9,11-octadecadienoic acid.

Diabetes is one of the most common metabolic diseases and affects hundreds of millions of individuals worldwide. There are two forms of diabetes mellitus: Type 1 (insulin-dependent) and Type II (non-insulin-dependent). The disease can lead to serious complications, including hyperglycemia, macroangiopathy, microangiopathy, neuropathy, nephropathy and retinopathy. Methods of treating diabetes have included administration of insulin in the

case of Type I diabetes and administration of various hypoglycemic agents in the case of Type II diabetes. Many of the known hypoglycemic agents exhibit undesirable side effects and are toxic in certain cases. Accordingly, there is a need for additional methods and compositions for treating diabetes. The present invention addresses this need.

SUMMARY OF THE INVENTION

It has been discovered that administration of CLA is advantageous in the treatment of diabetes mellitus. Accordingly, one preferred embodiment of the invention provides a method of treating diabetes including administering to an animal a therapeutically effective amount of CLA.

In a further aspect of the invention, it has been discovered that purified isomers of CLA can be used to advantage in the treatment of diabetes in animals. The invention thus provides methods involving the administration of purified CLA isomers to animals, alone or in predetermined admixtures, and food or administerable unit dosage forms (e.g., tablets, pills, etc.) containing such isomers or mixtures. In particular, a food composition is provided that includes a food product having a therapeutically effective amount of a purified isomer of CLA, such as cis,cis-9,11-octadienoic acid, trans,cis-10,12-octadecadienoic acid or a mixture of purified cis,trans-9,11-octadecadienoic acid and trans,cis-9,11-octadecadienoic acid.

Other features of the invention involve novel methods for modulating (e.g. increasing) the level of expression of certain genes, e.g. genes involved in regulating the expression of lipid metabolism enzymes and/or in regulating adipocyte differentiation, as illustrated in the Examples herein. The methods include administering to an animal an effective amount of CLA to modulate the gene expression.

It is an object of the invention to provide methods of treating an animal with diabetes by administering CLA.

It is a further object of the invention to provide food compositions that may advantageously be used for the treatment of diabetes mellitus.

These and other objects and advantages of the present invention will be apparent from the descriptions herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows the mechanism of action of peroxisome proliferators.

FIG. 2 depicts the biological effects of peroxisome-proliferator activated receptor (PPAR) activation by CLA.

FIG. 3 depicts graphs of the amount of chloramphenicol acetyltranferase produced as a percent of control versus the concentration of CLA and 100 μM of WY 14,643 with different PPAR subtypes. Left panel, PPARα; Middle panel, PPARβ; Right panel, PPARγ.

FIG. 4 represents bar graphs showing the extent that various CLA isomers activate the 3 different PPAR subtypes. All chemicals were given at 100 μM in dimethylsulfoxide (DMSO). Positive controls for PPARα (Wy 14,643), PPARβ (Bezafibrate; 2-[4-[2-[(4-chlorobenzoyl)amino]-ethyl]phenoxy]-2-methylpropanoic acid]) and PPARγ (Troglitazone) are shown for comparison. The furan used was 8-(5-hexyl-2-furyl)-octanoic acid which is an oxidation product of CLA. Data depicts the average of two experiments.

FIG. 5 represents bar graphs showing the extent that CLA and various CLA isomers activate full length PPARα. Panel A: shows activation of full length mouse PPARα by CLA. Transfected cells were treated for six hours with increasing concentrations of a CLA mixture (0 μM, 5 μM, 10 μM, 50 μM, 100 μM, 150 μM or 200 μM). Asterisks denote values that are significantly different from DMSO treated cells ($p<0.05$, $n = 3$); Panel B: shows activation of full length mPPARα by

different geometric isomers of CLA. Transfected cells were treated for six hours with 100 μM of each of the activators shown. Different letters denote significant differences ($p < 0.05$, $n=3$).

FIG. 6 represents a bar graph showing the extent that CLA and various CLA isomers activate full length mouse PPARβ. Transfected cells were treated with 100 μM of the indicated compounds. Asterisks denote significant differences ($p<0.01$, $n=3$).

FIG. 7 represents a bar graph showing the extent that CLA and various CLA isomers activate full length mouse PPARγ. Transfected cells were treated for six hours with 100 μM of the indicated compounds. Asterisks denote significant differences ($p<0.05$, $n=3$).

FIG. 8 depicts a bar graph showing the effects of CLA on markers of differentiation in 3T3-L1 preadipocytes. Mouse preadipoctye cells were treated at confluence for 48 hours with induction media which contains the indicated concentrations of CLA, 100 μM Wy 14,643 (Wy) or vehicle (DMSO). Induction media with insulin was subsequently added to the cells. Quantitative RT-PCR was performed using internal standards specific for each gene. The data is expressed as the average of three samples as a percent of DMSO treated cells correcting for β -actin expression.

FIG. 9 depicts a bar graph showing the effects of CLA and troglitazone (TZD) on tissue-specific gene expression. ACO and mAP2 were quantitated by RT-PCR. Asterisks denote a statistically significant difference from the rats fed the control diet ($P < 0.05$).

FIG. 10 represents graphs showing the effect of dietary CLA on glucose tolerance. Zucker lean (Panel A) or *fa/fa* (obese, Panel B) rats were fed experimental diets for 14 days and glucose tolerance was measured. Values represent mean glucose (mg/dl) ± S.D. (n = 4 lean rats or 8 fa/fa rats).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

For the purposes of promoting an understanding of the principles of the invention, reference will now be made to preferred embodiments and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications of the invention, and such further applications of the principles of the invention as illustrated herein, being contemplated as would normally occur to one skilled in the art to which the invention relates.

The present invention provides methods of treating diabetes and compositions useful in treating diabetes. In one aspect of the invention diabetes is treated in an animal by administering a therapeutically effective amount of CLA. Administration of CLA advantageously normalizes glucose tolerance in diabetic animals as well as reduces plasma insulin, triglyceride and free fatty acid levels. Although the method is advantageous in treating Type II (non-insulin-dependent) diabetes mellitus, it may also be used to treat Type I (insulin-dependent) diabetes mellitus in conjunction with other treatments therefor as known in the art. In yet another aspect of the invention, methods and compositions are provided which involve the use of purified CLA isomers or purified mixtures of CLA isomers. The compositions may include, and the methods may involve the use of, a therapeutically effective amount of purified cis,cis-9,11-octadecadienoic acid,

purified trans,cis-10,12-octadecadienoic acid, a mixture of purified cis,trans-9,11-octadecadienoic acid and trans,cis-9,11-octadecadienoic acid, or another purified isomer of CLA.

In a first aspect of the invention, a method of treating diabetes in an animal is provided that includes administering to the animal a therapeutically effective amount of CLA, including salts thereof, esters thereof (including, for example, monoglycerides, diglycerides and triglycerides) active isomers thereof and mixtures thereof. CLA refers to a group of positional and geometric isomers of linoleic acid (cis,cis-9,12-octadecadienoic acid). The positional isomers include isomers having double bonds at either carbon atoms 9 and 11 or carbon atoms 10 and 12 whereas the geometric isomers include isomers having the cis and/or trans configuration. Thus, there are several possible isomers of CLA, including, but not limited to: cis,cis-9,11-octadecadienoic acid; cis,trans-9,11-octadecadienoic acid; trans,cis-9,11-octadecadienoic acid; trans,trans-9,11-octadecadienoic acid; cis,cis-10,12-octadecadienoic acid; cis,trans-10,12-octadecadienoic acid; trans,cis-10,12-octadecadienoic acid; and trans,trans-10,12-octadecadienoic acid. The cis,trans-9,11 and trans,cis-9,11 isomers have not yet been isolated independently from each other and the literature loosely uses the term cis,trans-9,11-octadecadienoic acid to refer to both the cis,trans-9,11 and the trans,cis-9,11 isomers.

The CLA utilized in the present invention may be prepared using techniques known to the art and

literature or may be obtained as a commercial product. CLA may be obtained commercially, for example, from companies such as Pharmanutrients, Inc., Lake Bluff, IL; NuChek Prep, Elysian MN; and Peak Nutrition, Syracuse, NE. However, the CLA sold by NuCheck Prep is preferred. The relative proportions of the isomers may vary in the commercially available CLA. The commercial composition may also include other fatty acids such as linoleic acid as well as other lipids such as straight chain hydrocarbons having polar end groups. For example, the CLA mixture may include other fatty acids known in the art, saturated or unsaturated, or breakdown products of CLA. The commercial composition may also include antioxidants such as vitamin E, butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT). CLA may also be synthesized by methods known in the art. For example, CLA may be synthesized from isomerization of linoleic acid utilizing, for example, a radical-generating species and a protein rich in sulfur residues as known in the art and as described in Dormandy TL, Wickens DG, *Chem. Phys. Lipids* 45:353-64 (1987) which is hereby incorporated by reference in it entirety. As another example, CLA may be synthesized from either linoleic acid or safflower oil by heating the linoleic acid or safflower oil in an inert atmosphere with subsequent acidification and extractions as described in U.S. Patent No. 5,670,082 to Cook et al. which is hereby incorporated by reference in its entirety. Moreover, specific isomers of CLA, such as the trans,trans 9-11, the cis,cis-9,11 isomer, the cis,trans-9,11 (in

combination with the trans,cis-9,11 isomer) and the cis,trans-10,12 isomers can be currently synthesized in pure form by methods known in the art. The salts of CLA are those known in the art, including the sodium and potassium salts.

Linoleic acid used to synthesize CLA, or other fatty acids included in the mixture, may be obtained from plant sources, including soybean, cottonseed, corn, sunflower, safflower, canola and palm oils. Soybean, corn, sunflower and safflower oil are particularly rich in linoleic acid. Linoleic acid may also be obtained from hydrolysis of triglycerides isolated from plant sources by methods known in the art. For example, triglycerides may be obtained from plant sources by solvent extraction of plant biomass using aliphatic solvents. Subsequent additional purification may involve distillation, fractional crystallization, degumming, bleaching and steam stripping. The triglycerides may be hydrogenated as needed. The triglycerides may then be hydrolyzed either by enzymatic (e.g., use of lipase) or chemical methods (e.g., by alkaline hydrolysis) known in the art. Linoleic acid may also be synthesized from petrochemical fatty alcohols. Alternatively, free fatty acids and triglycerides may be obtained from commercial sources, including Cargill, Archer Daniel Midlands and Central Soya.

CLA may also be found in ruminant meats, pasteurized dairy products and processed cheeses. Moreover, the amount of CLA in dairy products may be increased by methods known in the art. For example,

the amount of CLA in cow's milk may be increased by feeding to a lactating cow a diet either solely of grass or one which contains about 1% to about 5% by weight of a vegetable oil containing linoleic acid or linolenic acid as described in U.S. Patent No. 5,770,247 to Satter et al. which is hereby incorporated by reference in its entirety. CLA may also be obtained by enzymatic conversion of linoleic acid as known in the art. For example, CLA may be prepared utilizing the enzyme W[illegible]-cis,W[illegible]-transisomerase. The enzyme may be obtained, for example, from rumen bacteria, such as *Butyrivibrio fibrisolvens*. Harmless microorganisms in the intestinal tracts of rats and other monogastric animals may also convert linoleic acid to CLA as described in Chin, SF et al., FASEB J, 6 (1992).

CLA may be administered in various forms. For example, CLA may be administered in tablet form, in a solution or emulsion, or in a capsule. CLA may also be mixed with a pharmaceutically acceptable carrier. In tablet form, a solid carrier may include, for example, lactose, starch, carboxymethyl cellulose, dextrin, calcium phosphate, calcium carbonate, synthetic or natural calcium silicate, magnesium oxide, dry aluminum hydroxide, magnesium stearate, sodium bicarbonate, dry yeast or a combination thereof. In solution, the carrier may be an oil but is preferably sterile water or a sterile saline solution for parenteral administration. CLA may also be administered in forms in which other drugs known in the art are administered.

CLA may be administered in a variety of ways. For example, CLA may be administered parenterally, such as

orally, intravenously, rectally, as well as intraperitoneally.

In another feature of the invention, it has been discovered that certain CLA isomers have higher activity. Accordingly, in yet another aspect of the invention, purified CLA isomers may be administered to animals in need thereof and may be added to a food product to form a food composition. The CLA isomers may be added to a food product in any form, such as a powder or in an oil such as corn oil either alone or with another oil, such as coconut oil. One preferred food composition includes CLA predominantly (i.e., greater than 50%) comprised of a mixture of purified cis,trans-9,11-octadecadienoic acid and trans,cis-9,11-octadecadienoic acid. Another beneficial food composition may include a mixture predominantly comprised of cis,cis-9,11-octadecadienoic acid or trans,cis-10,12-octadecadienoic acid. In a further preferred embodiment, the food composition may include a mixture of purified cis,trans-9,11-octadecadienoic acid and trans,cis-9,11-octadecadienoic acid. In this regard, the term "purified" as used herein to refer to a particular CLA isomer or mixture of isomers means a CLA composition containing no more than about 10% by weight of CLA isomers other than those specified. Preferably, the identified isomer or mixture will contain no more than about 5% by weight and more preferably no more than about 3% by weight of the other CLA isomers. In yet other aspects of the invention, the food composition may include purified cis,cis-9,11-octadecadienoic acid, or other purified CLA isomers,

including trans,cis-10,12-octadecadienoic acid. In further embodiments, the food composition may include a purified mixture of CLA. For example, CLA may be purified to different extents to produce a purified mixture of CLA including less than all of the CLA isomers. The purified CLA isomers may be included in any food product, including, for example, cereals, meats, eggs, cheeses and other dairy products, vegetables, breads and other flour or bran-based products, and confection products. The CLA isomers may also be added to any consumable liquid but may require various emulsifying agents for dissolution.

The therapeutically effective amount administered will have a beneficial effect on an animal with diabetes. For example, the therapeutically effective amount is desirably sufficient to normalize glucose tolerance in a diabetic animal. Normalization of glucose tolerance can be determined, for example, by a glucose tolerance test as known in the art and as described in the examples below. Moreover, the amount of CLA administered will also preferably be sufficient to reduce blood levels of insulin and/or to reduce the level of circulating free fatty acids or triglycerides. The blood levels of insulin, free fatty acids, and triglycerides are desirably reduced by at least about 5%, more preferably by at least about 20%, and further most preferably by at least about 50%. The amount of CLA administered to an animal with diabetes will vary depending on the age of the animal, the general health of the animal and the severity of their diabetic condition. However, it is expected that an animal

being treated for diabetes will usually receive at least about 1 mg CLA/kg body weight/day up to the highest level which is not toxic to the animal. Typically, an animal may receive about 1 mg CLA/kg body weight/day up to about 10,000 mg CLA/kg body weight/day. However, it is expected that relatively low doses of CLA will be sufficient, for instance, falling in the range of about 1 mg CLA/kg body weight/day to about 150 mg CLA/kg body weight/day and more desirably about 10 mg CLA/kg body weight/day to about 50 mg CLA/kg body weight/day. Furthermore, when the CLA is included in a food product, it is advantageous to include an amount of CLA per serving of food product that will provide the preferred amounts of CLA/kg body weight/day discussed above.

In yet another feature of the invention, CLA may be administered to an animal in a composition that releases CLA internally, for example, in the form of an ester of CLA, preferably a triglyceride. In a further preferred embodiment, the triglyceride includes at least one CLA residue in the form of an ester with glycerol and may have other unsaturated or saturated fatty acid residues, but preferably the unsaturated fatty acid linoleic acid. In a more preferred aspect, the triglyceride includes three CLA residues in the form of an ester with glycerol. The CLA residues are preferably the most active isomers of CLA, such as the cis,trans-9,11 and trans,cis-9,11 isomer or the cis,cis-9,11 isomer, but may include any of the other isomers. Upon ingestion, the CLA residues may be released in the stomach of the animal by enzymatic

hydrolysis through, for example, the action of a lipase. The triglycerides may be purified from plant sources as described above, may be purchased commercially or may be synthesized from glycerol and the respective fatty acids by methods known in the art. The therapeutically effective amount that is administered will be dependent on at least the factors discussed above. The amount of triglyceride that is administered may be that which provides the amount of CLA specified above. The amount of triglyceride required to achieve a specific dose will depend on the number of CLA esters or residues comprising the triglyceride and can be easily calculated by one skilled in the art. The triglyceride may be administered in similar forms as described above for CLA.

CLA may be administered to an animal with diabetes, including warm-blooded vertebrates such as mammals. The list of mammals includes, for example, humans.

Reference will now be made to specific examples illustrating the compositions and methods above. It is to be understood that the examples are provided to illustrate preferred embodiments and that no limitation to the scope of the invention is intended thereby. Data from the studies below were analyzed by ANOVA (General Linear Model, LSD) using Statistical Analysis System (SAS; Cary, NC) or StatView for the Macintosh (Abacus Concepts, Berkeley, CA).

EXAMPLE 1

Activation of Peroxisome Proliferator-Activated Receptor (PPAR) by CLA

In this example, CLA is shown to be involved in the activation of several PPAR subtypes. PPAR, an intracellular protein receptor, is a member of the steroid hormone superfamily that may be important in regulating the expression of lipid metabolism enzymes and may have an effect on cell growth and/or differentiation. Three subtypes of PPAR (α, β and γ) have been identified in several species, including human. PPARγ is thought to be involved in the anti-diabetic and glucose lowering activity of groups of drugs known as thiazolidinediones and fibrate hypolipidemic drugs. PPAR can be activated by peroxisome proliferators, thiazolidinediones and fatty acids. The mechanism of action of peroxisome proliferators is depicted in FIG. 1 and the effects of the activators of PPAR subtypes is shown in Table 1.

Table 1. Activators of PPAR subtypes and their effects.

Drug/Chemical Group	PPARα	PPARβ	PPARγ	Clinical Use or *effects*
Peroxisome proliferators	++++	+−	+++	Hypolipidemia, possible antidiabetic, *hepatic peroxisome proliferation, adipocyte differentiation*
Long-chain fatty acids	+++	+	++	*Hypolipidemia, hepatic peroxisome proliferation, adipocyte differentiation*
Thiazolidinediones	-	-	++++	**Antidiabetic**, *adipocyte differentiation, decreased insulin resistance, decreased blood glucose levels*
CLA	+++	+	++	*Anti-cancer effects, anti-atherogenic effects, hypolipidemia, hepatic peroxisome proliferation,* ***Antidiabetic*** *as shown in this disclosure*

COS-1 cells (American Type Culture Collection) were maintained in α-minimal essential media (Sigma)

supplemented with 8% fetal calf serum (Gibco BRL), 0.2 mg/ml streptomycin and 200 U/ml penicillin. The pSG5-GAL4-PPAR chimera expression constructs, containing the ligand binding domain of mouse PPARα, β or γ, as well as the $(UAS)_5$-tk-CAT reporter construct were kindly provided by Steven A. Kliewer (Glaxo Research Institute). At 75-90% confluence, COS-1 cells were co-transfected with GAL4-PPAR, $(UAS)_5$-tk-CAT, and pSV-βGal (Promega) as described in Lehmann, J.M. et al., *J.Biol.Chem.* 270, 12953-12956 (1995). Twenty-four hours after transfection, the cells were treated with the indicated amounts of CLA, or a single 100 μM dose of 4-chloro-6-(2,3-xylindino)-2-pyrimidinylthio)-acetic acid (Wy 14,643; a hypolipidemic drug known as a peroxisome proliferator). After 6 hours of treatment, the cells were harvested and chloramphenicol acetyltransferase levels were assessed by ELISA (Gibco BRL) according to the manufacturer's instructions. Data is expressed relative to β-galactosidase activity. CLA used in this experiment was obtained from a commercially available mixture from NuChek Prep, Elysian MN. The mixture contained about 41.2% by weight of a composition including cis,trans-9,11-octadecadienoic acid and trans,cis-9,11-octadecadienoic acid, about 44% by weight trans,cis-10,12-octadecadienoic acid, about 9.4% by weight cis,cis-10,12-octadecadienoic acid, about 1.3% by weight of a composition including trans,trans-9,11-octadecadienoic acid and trans,trans-10,12-octadecadienoic acid, about 1.1% by weight cis,cis-9,11-octadecadienoic acid, about

0.7% by weight linoleic acid and about 2.2% of other lipids as mentioned above.

FIG. 2 shows that all subtypes of PPAR studied were activated by CLA. PPARα was activated to a greater extent than either PPARβ or PPARγ. However, PPARβ and PPARγ were activated a significant amount (approximately 2-fold more than the control value). The activation of PPARα by the commercially available mixture is believed to be the result of the cis,trans-9,11-octadecadienoic acid isomer as discussed in Example 2. Moreover, the biological effects of PPAR activation by CLA will depend on the tissue and the predominant PPAR subtype being examined as shown in FIG. 3.

EXAMPLE 2

Activation of PPAR Subtypes by CLA Isomers

In this example, certain PPAR subtypes are shown to be activated by CLA isomers. The same experimental procedure as described in Example 1 was carried out to generate the data shown in FIG. 4. However, a 100 μM concentration of selected isomers of CLA were also utilized in the transfection assay to determine whether specific isomers of CLA could activate any of the PPAR subtypes.

The data in FIGS. 5-7 was generated utilizing constructs including full length mouse PPARα, PPARβ or PPARγ and a luciferase reporter gene. The CV-1 cell line (African green monkey kidney cells) used was obtained from American Type Culture Collection (#CCL-70). The cells were grown in Eagle minimal essential

medium containing 10% fetal bovine serum (GIBCO). For each transfection involving PPARα, 625 ng pcDNA3-PPARα expression vector was used along with 250 ng of psV-GL-2-PPRE-luciferase reporter plasmid and 250 ng of pSV-β-galactosidase internal control plasmid. For each transfection involving PPARβ or PPARγ, either 625 ng pSG5-mouse-PPARβ or 625 ng pSG5-mouse-PPARγ was used along with 250 ng of the psV-GL2-PPRE-luciferase reporter plasmid and 250 ng of pSV-β-galactosidase internal control plasmid. Cells were transfected using Lipofect AMINE™ reagent (GIBCO) and phenol red-free, serum free medium (OptiMEM©I, GIBCO Life Technologies, Grand Island, NY). Seven hours post-transfection, charcoal stripped serum (Cocalico Biologicals, Inc. Reamstown, PA) was added to the media (10% final concentration) for an overnight incubation (16 hours). Transfected cells were treated for six hours with various doses or 100 μM of CLA, the 9Z,11E (cis,trans-9,11) isomer(97% purity), the 9E,11E (trans,trans-9,11) isomer (98% purity), the 10E,12Z (trans,cis-10,12) isomer or the other indicated activators. Luciferase and β-galactosidase activities were assayed on cell lysates following the manufacturer's protocols (Promega, Madison, WI). The data were quantified relative to luciferase/β-galactosidase activity expressed as a ratio to vehicle-treated cells (0.1% DMSO).

FIG. 4 shows that all of the isomers examined activated all of the PPAR subtypes. However, the 9Z11Z (cis,cis-9,11) and 9Z11E (cis,trans-9,11) isomers activated PPARα and PPARβ more than the CLA mixture and

the 9E11E (trans,trans-9,11) isomer only activated PPARβ more than CLA mixture alone. None of the isomers activated PPARγ more than the CLA mixture. Moreover, in a similar study, human PPARγ was also activated by CLA (data not shown), showing that the molecular events underpinning the present invention are also occurring in humans.

The data shown in FIGS. 5-7 show that all of the CLA isomers tested, including the trans,cis-10,12-octadecadienoic acid isomer, activate the respective PPAR subtypes with respect to the DMSO control. Moreover, the data in FIGS. 5 and 6 further show that the trans,cis-10,12 CLA isomer activated PPARα and PPARβ significantly more than the CLA mixture alone.

EXAMPLE 3

Effect of CLA on Gene Expression

Activation of certain PPAR subtypes results in altered gene expression, such as gene induction. In this example, CLA was found to induce two markers of differentiation of mouse 3T3-L1 preadipocytes into differentiated adipocytes, which requires PPARγ activation. The two markers studied were adipocyte protein-2 (mAP2) mRNA and PPARγ mRNA.

3T3-L1 Cell Culture

Mouse 3T3-L1 preadipocytes (American Type Culture Collection) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Gibco BRL) 0.2 mg/ml streptomycin and 200 U/ml

penicillin ("growth media"). Differentiation was induced as described by Brandes, R., Arad R., and Bar-Tana, J., *Biochem. Pharmacol.* 50, 1949-1951 (1995). Briefly, differentiation was induced by adding various concentrations of CLA (25-250 μM final concentration), linoleic acid (100μM), Wy 14,643 (100μM) or vehicle (DMSO) in DMEM with 10% FCS and 0.1 μM dexamethasone ("induction media") to confluent 3T3-L1 preadipocytes. After 48 hours, the induction media was removed and replaced by induction media with 4 mU/ml insulin. This media was changed every 48 hours. At various time intervals, the cells were rinsed twice with PBS and total RNA extracted using TriReagent (Molecular Research Center).

The differentiation of mouse 3T3-L1 cells was monitored by examining adipocyte-specific markers including PPARγ (γ1 and γ2) and adipocyte protein-2 (mAP2). The housekeeping gene β-actin was also examined as described in Vanden Heuvel, J.P. et al., *Cancer Res.* 54, 62-68 (1994). Quantitative reverse transcriptase polymerase chain reaction was utilized to determine mRNA expression for these genes (as described in Vanden Heuvel, J.P., *PCR Applications in Molecular Toxicology*, 218 pgs. CRC Press, Boca Raton, FL (1997), see Table 2 for primer sequences utilized) using internal standards specific for each primer set (as described in Vanden Heuvel, J.P., Tyson, F. and Bell, D.A., *Biotechniques* 14, 395-398 (1993)).

Table 2. Sequence of primers utilized in RT-PCR

Primer	Sequence	Length of Product (bp) Target	Int. Std.*
mAP2 forward	5' ACT GTG GCC TGA GCG ACT TCT ATG	190	314
mAP2 reverse	5' AGG GGG CTT CTG GCA AAC AAT		
mPPARγ forward	5' TGC TGG CCT CCC TGA TGA ATA	315	352
mPPARγ reverse	5' TTG GCG AAC AGC TGA GAG GAC		
Actin forward	5' CCT CTA TGC CAA CAC AGT	125	153
Actin reverse	5' AGC CAC CAA TCC ACA CAG		
ACO forward	5' ATT CGG TGT TGT AAG TGC	417	340
ACO reverse	5' TTG GTG GGT GGG TGT TGA		

* An internal standard was synthesized only for genes that were to be quantitated

As seen in FIG. 8, CLA is effective at inducing both mAP2 and PPARγ mRNA. It is also seen that CLA is more potent as a PPARγ ligand in the 3T3-L1 bio-assay than would have been expected from the transactivation assays, the results of which are depicted in FIG. 4. FIG. 8 also shows that the most effective concentration of CLA in the differentiation assay was 50 μM.

Animal Studies

Male Zucker fatty (*fa/fa*) rats and lean littermates (wt) were obtained at six weeks of age from Genetic Models, Inc. (Indianapolis, IN). Because the primary aim of the study was to determine the ability of CLA to improve insulin action and prevent the onset of diabetes, all rats were determined normoglycemic prior to assignment to experimental treatments. (The diets are discussed in the subsequent section). After maintaining rats on experimental diets for 14 days, rats were euthanized by CO_2 and cervical dislocation and tissues collected, weighed and frozen. RT-PCR was performed as described above.

The genes utilized as markers of tissue and subtype specific PPAR activation included Acyl-CoA Oxidase

(ACO; found in the liver and induced by PPARα activation), Adipocyte Specific Protein (mAP2; found in adipose tissue and induced by activation of PPARγ) and ACO in the muscle (induced by PPARβ).

As seen in FIG. 9, both CLA and Troglitazone (5-[[4-[3,4-Dihydro-6-hydroxy-2,5,-7,8-tetramethyl-2H-1-benzopyran-2-yl)methoxy]phenyl]methyl]-2,4-thiazolidinedione; TZD; Rezulin, Parke-Davis) significantly induce ACO mRNA expression in the PPARα-containing tissue (liver) and a tissue with predominantly PPARγ (adipose tissue) but had no effect on a tissue with predominantly PPARβ (muscle). The induction of mAP2 in adipose tissue verifies the PPARγ activation observed in the 3T3-L1 cells.

EXAMPLE 4

Effect of Dietary CLA on Normalizing Glucose Tolerance in the Zucker Fatty *fa/fa* Rat

The Zucker *fa/fa* rats are an excellent animal model for the examination of adult onset diabetes. In this example, the effect of three different diets (control, CLA, TZD) on the levels of circulating insulin, triglycerides and free fatty acids in the fa/fa rats as well as their lean counterparts (wildtype, wt) were determined. Moreover, to determine if CLA increases insulin sensitivity as a PPARγ activator, such as TZD, a glucose tolerance test was performed.

Diet components were obtained from Dyets, Inc. (Bethlehem, PA) and the CLA isomeric mixture (90% pure mixture) from PharmaNutrients, Chicago, IL. The CLA

mixture had the following isomeric distribution: 42% of a composition including cis,trans-9,11 and trans,cis-9,11-octadecadienoic acid; 43.5% trans,cis-10,12-octadecadienoic acid; 1% cis,cis-9,11-octadecadienoic acid; 1% cis,cis-10,12-octadecadienoic acid; and 1.5% of a composition including trans,trans-9,11-octadecadienoic acid and trans,trans-10,12-octadecadienoic acid, all on a weight percent basis. The CLA mixture also included, on a weight percent basis, about 0.5% linoleate, about 5.5% oleate and about 5% other lipids as discussed above. The thiazolidinedione, TZD (Rezulin™, Parke-Davis, Ann Arbor, MI), was used as a positive control for anti-diabetic activity in these studies. Male Zucker fatty (*fa/fa*) rats and lean littermates (wt) were obtained at six weeks of age from Genetic Models, Inc. (Indianapolis, IN). Because the primary aim of the study was to determine the ability of CLA to improve insulin action and prevent the onset of diabetes, all rats were determined normoglycemic prior to assignment to experimental treatments. After maintaining rats on experimental diets for 14 days, rats were euthanized by CO_2 and cervical dislocation and blood collected and immediately analyzed for post-prandial glucose concentrations (see below) or placed into heparinized test tubes for plasma analyses as described below. Epididymal fat pads and livers were harvested and weighed. An aliquot of the epididymal fat pad was isolated into buffered saline for glucose transport analyses and the remaining epididymal fat pad and gastrocnemius muscle were isolated, immediately frozen

in liquid nitrogen and stored at -80°C until mRNA and protein analyses were performed.

Experimental Diets

Three isocaloric, experimental diets were formulated according to a modified AIN-76 mixture containing 6.5% (by weight) fat (diet described in American Institute of Nutrition: Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies, *J. Nutr.* 107 1340-1348 (1977) but includes 6.5% by weight fat instead of 5% by weight fat). The same amount of corn oil (5%) was used in all diets since corn oil is rich in linoleic acid, an essential fatty acid. The diets contained either 5% corn oil + 1.5% lard + no CLA (Control Diet), 5% corn oil + 1.5% CLA (CLA Diet), or 5% corn oil + 1.5% lard + 0.2% troglitazone (TZD Diet). A dose of 1.5% CLA was chosen based on previous studies in our laboratory showing this dose to modulate PPAR-associated gene expression in the liver (Belury, M.A. et al., *Nutr.Biochem.* 8:579-84 (1997)) and inhibit tumorigenesis in murine skin (as shown in Belury, M.A. et al., *Nutr. Cancer* 26, 149-157 (1996)). The dose of TZD (0.2%) used in this study has been shown to be effective at normalizing glucose tolerance after 15 days and suppressing elevated glucose, triglycerides, free fatty acids and urinary protein in Zucker (*fa/fa*) rats. Diets were fed on alternate days and rats were allowed free access to food and water. Body weights were measured twice weekly and average food consumption estimated by measuring differences in weight of freshly supplied diet and diet remaining in feeders two days

later. Taking into account the average body weight of the *fa/fa* rats and the amount of food they consumed, the *fa/fa* rats received a daily dose of about 1.71 mg CLA/kg body weight, which amounted to a daily dose of about 375 mg.

Glucose Tolerance Tests

In order to compare the effects of CLA and TZD on insulin action, a glucose tolerance test was conducted on day 11 of dietary intervention. Animals were fasted overnight (16 hours). Conscious rats were injected intraperitoneally with D-glucose (1 g/kg body weight) and blood samples were collected via the tail vein prior to the injection (time 0) and at 2, 5, 10, 15, 20, 40, 60, 120 and 180 minutes following injection.

Determination of Blood Metabolite and Hormone Concentrations

Blood glucose levels were determined using a One Touch glucose meter (Lifescan, Inc.). Plasma insulin levels were determined using commercially available radioimmunoassay kits (Linco Research, St. Charles, MO). Plasma nonesterified fatty acids were quantified using a colorimetric kit (Wako). Plasma triglyceride concentrations were determined using a commercially available kit (Sigma Diagnostics, St. Louis, MO).

FIG. 10 depicts the results of the glucose tolerance test. As expected, a decreased ability to remove glucose from the blood is seen in the *fa/fa* rats (compare lean control versus obese control). In the *fa/fa* rats fed either CLA or TZD, blood glucose was reduced much more rapidly than the respective control animals. As glucose tolerance is the predominant test used to assess the existence of non-insulin-dependent

diabetes mellitus (NIDDM), the data depicted in FIG. 10 convincingly show that CLA is as effective as TZD for improving glucose tolerance. Therefore, CLA may be an effective treatment for individuals with NIDDM.

The results showing the relative levels of circulating insulin, plasma triglycerides and circulating free fatty acids are shown in Table 3.

Table 3. Effect of Dietary CLA on Glucose, Triglyceride and Free Fatty Acid Concentrations in Zucker Rats[*]

Diet	Insulin (ng/dl) ± S.D.	Plasma Triglycerides (mg/dl) ± S.D.	Free Fatty Acids (mMol) ± S.D.
wt, Control	2.8±0.1[a]	92.1±16.7[bc]	1.651±0.497[ab]
wt, CLA	2.8±0.5[a]	66.2±18.0[bc]	1.170±0.335[bc]
wt,TZD	1.4±0.1[a]	61.1±12.1[c]	1.139±0.277[c]
fa/fa, Control	38.9± 2.8[b]	408.3±148.7[a]	1.959±0.402[a]
fa/fa, CLA	20.6±3.3[c]	149.4±78.4[b]	1.004±0.262[c]
fa/fa, TZD	5.6±0.5[d]	57.08±12.3[c]	0.778±0.378[c]

[*] Plasma insulin triglycerides and free fatty acid concentrations were measured in fed rats after experimental diets were fed for 14 days.
[a-d] Values (± S.D.) with significant differences ($p<0.05$) within columns are denoted by different superscripts.

As expected, the *fa/fa* rats exhibited higher plasma insulin and triglycerides compared to wt animals. However, CLA significantly improved symptoms of diabetes causing a 50-60% decline in plasma insulin, triglycerides and free fatty acids. Moreover, TZD markedly decreased circulating insulin, triglycerides and free fatty acids in the *fa/fa* rats, thus verifying TZD as an effective anti-diabetic agent. For additional information on the normalization of glucose

tolerance and other biological effects using CLA, reference may be made to Biochem. Biophys. Res. Comm., **244**, 678-682 (1998).

While the invention has been illustrated and described in detail in the drawings and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiment has been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected. In addition, all references cited herein are indicative of the level of skill in the art and are hereby incorporated by reference in their entirety.

What is claimed is:

1. A method of treating diabetes in an animal, said method comprising administering to said animal a therapeutically effective amount of conjugated linoleic acid.

2. The method of claim 1, wherein said conjugated linoleic acid is administered orally.

3. The method of claim 2, wherein said conjugated linoleic acid is administered in a unit dosage form.

4. The method of claim 3, wherein said unit dosage form is a food product.

5. The method of claim 1, wherein said conjugated linoleic acid is selected from the group consisting of 9,11-octadecadienoic acid, esters thereof, geometric isomers thereof, salts thereof and mixtures thereof.

6. The method of claim 5, wherein said geometric isomers have configurations selected from the group consisting of trans,trans; cis,cis; trans,cis; and cis,trans.

7. The method of claim 1, wherein said conjugated linoleic acid is selected from the group consisting of 10,12-octadecadienoic acid, esters

thereof, geometric isomers thereof, salts thereof and mixtures thereof.

8. The method of claim 7, wherein said geometric isomers have configurations selected from the group consisting of trans,trans; cis,cis; trans,cis; and cis,trans.

9. The method of claim 1, wherein said CLA is comprised predominantly of cis,trans-9,11-octadecadienoic acid and trans,cis-9,11-octadecadienoic acid.

10. The method of claim 1, wherein said CLA is comprised predominantly of cis,cis-9,11-octadecadienoic acid.

11. The method of claim 1, wherein said conjugated linoleic acid is administered in an amount of about 1 mg of said conjugated linoleic acid/kg body weight to about 10,000 mg of said conjugated linoleic acid/kg body weight.

12. The method of claim 1, wherein said animal is a mammal.

13. The method of claim 12, wherein said mammal is a human.

14. The method of claim 1, wherein said conjugated linoleic acid is administered in a pharmaceutically acceptable carrier medium.

15. The method of claim 14, wherein said pharmaceutically acceptable carrier medium includes water.

16. A food composition useful in treating diabetes comprising, a food product having a therapeutically effective amount of conjugated linoleic acid, said conjugated linoleic acid predominantly comprised of a mixture of cis,trans-9,11-octadecadienoic acid and trans,cis-9,11-octadecadienoic acid.

17. The food composition of claim 16, wherein said therapeutically effective amount of said mixture is sufficient to provide about 1 mg of said mixture/kg body weight per serving to about 10,000 mg of said mixture/kg body weight per serving.

18. A food composition useful in treating diabetes comprising, a food product having a therapeutically effective amount of conjugated linoleic acid, said conjugated linoleic acid predominantly comprised of cis,cis-9,11-octadecadienoic acid.

19. The food composition of claim 18, wherein said conjugated linoleic acid is administered in an amount sufficient to provide about 1 mg of said cis,cis-9,11-octadecadienoic acid/kg body weight per serving to about 10,000 mg of said cis,cis-9,11-octadecadienoic acid /kg body weight per serving.

20. A food composition useful in treating diabetes comprising, a food product having a therapeutically effective amount of conjugated linoleic acid, said conjugated linoleic acid predominantly comprised of trans,cis-10,12-octadecadienoic acid.

21. The food composition of claim 20, wherein said conjugated linoleic acid is administered in an amount sufficient to provide about 1 mg of said trans,cis-10,12-octadecadienoic acid/kg body weight per serving to about 10,000 mg of said trans,cis-10,12-octadecadienoic acid /kg body weight per serving.

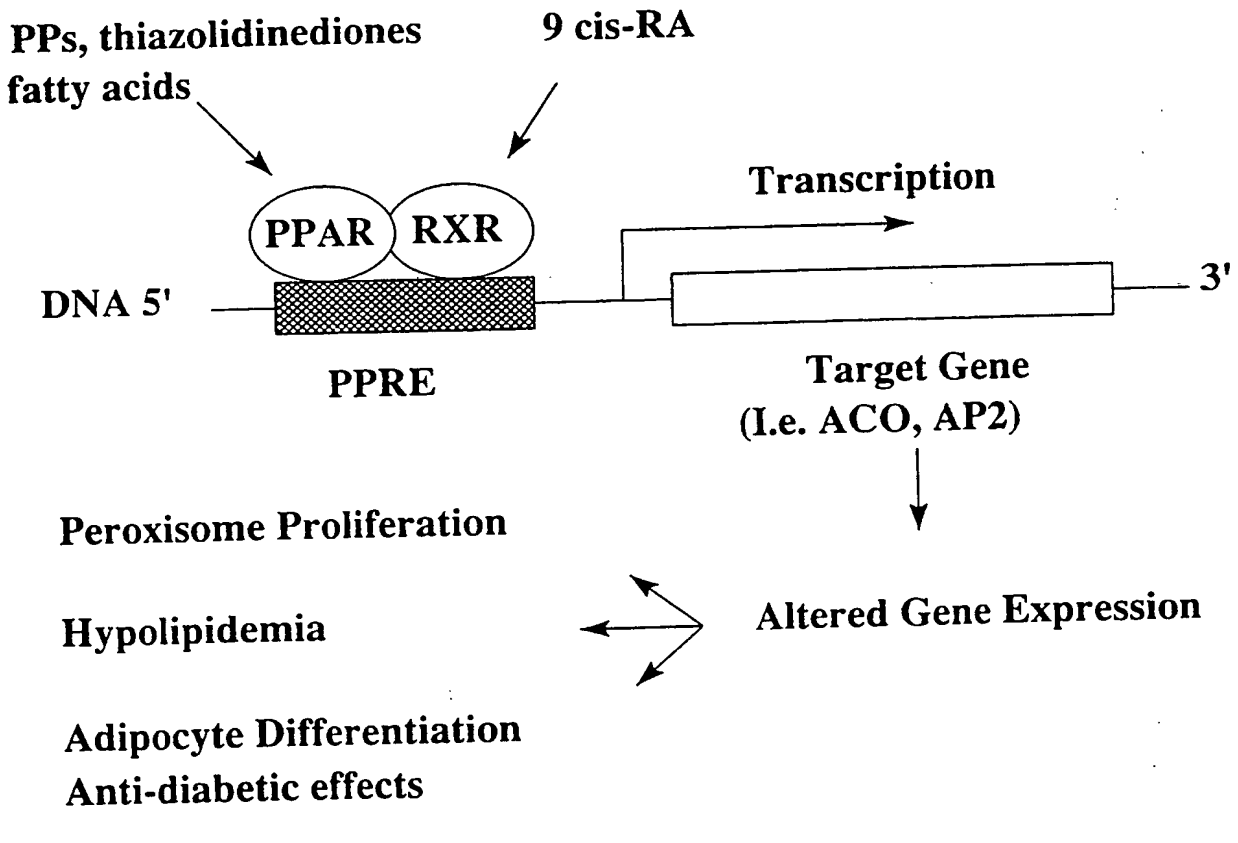

PPs, thiazolidinediones
fatty acids
9 cis-RA
Transcription
PPAR
RXR
DNA 5'
3'
PPRE
Target Gene
(I.e. ACO, AP2)
Peroxisome Proliferation
Hypolipidemia
Altered Gene Expression
Adipocyte Differentiation
Anti-diabetic effects

Fig. 1

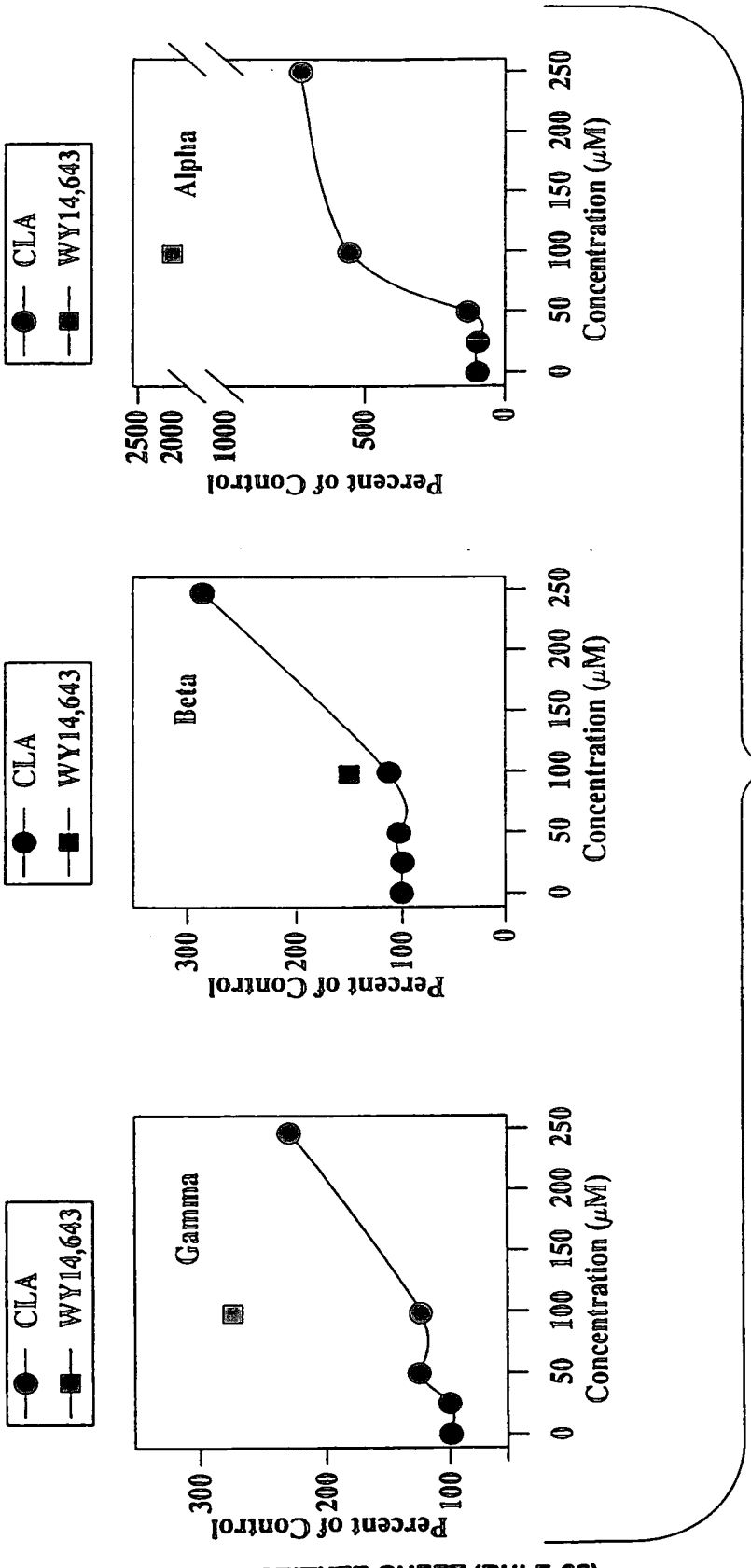

CLA
WY14,643
Gamma
Beta
Alpha
Percent of Control
Concentration (μM)
0
50
100
150
200
250
100
200
300
500
1000
2000
2500

Fig. 2

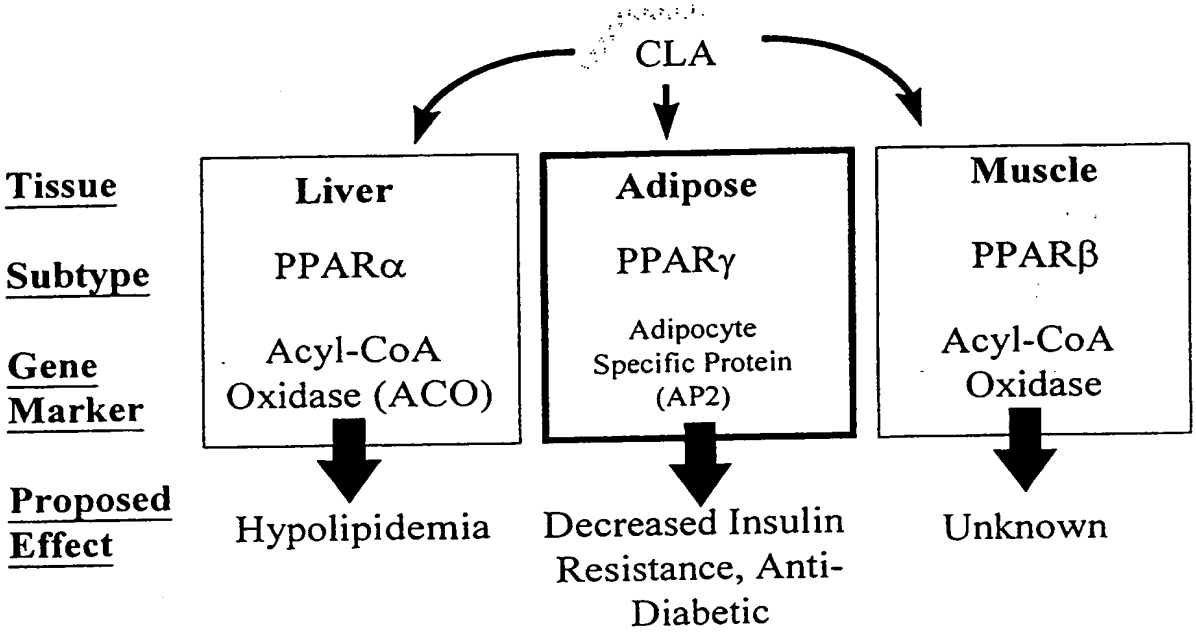

CLA
Tissue
Subtype
Gene Marker
Proposed Effect
Liver
PPARα
Acyl-CoA Oxidase (ACO)
Hypolipidemia
Adipose
PPARγ
Adipocyte Specific Protein (AP2)
Decreased Insulin Resistance, Anti-Diabetic
Muscle
PPARβ
Acyl-CoA Oxidase
Unknown

Fig. 3

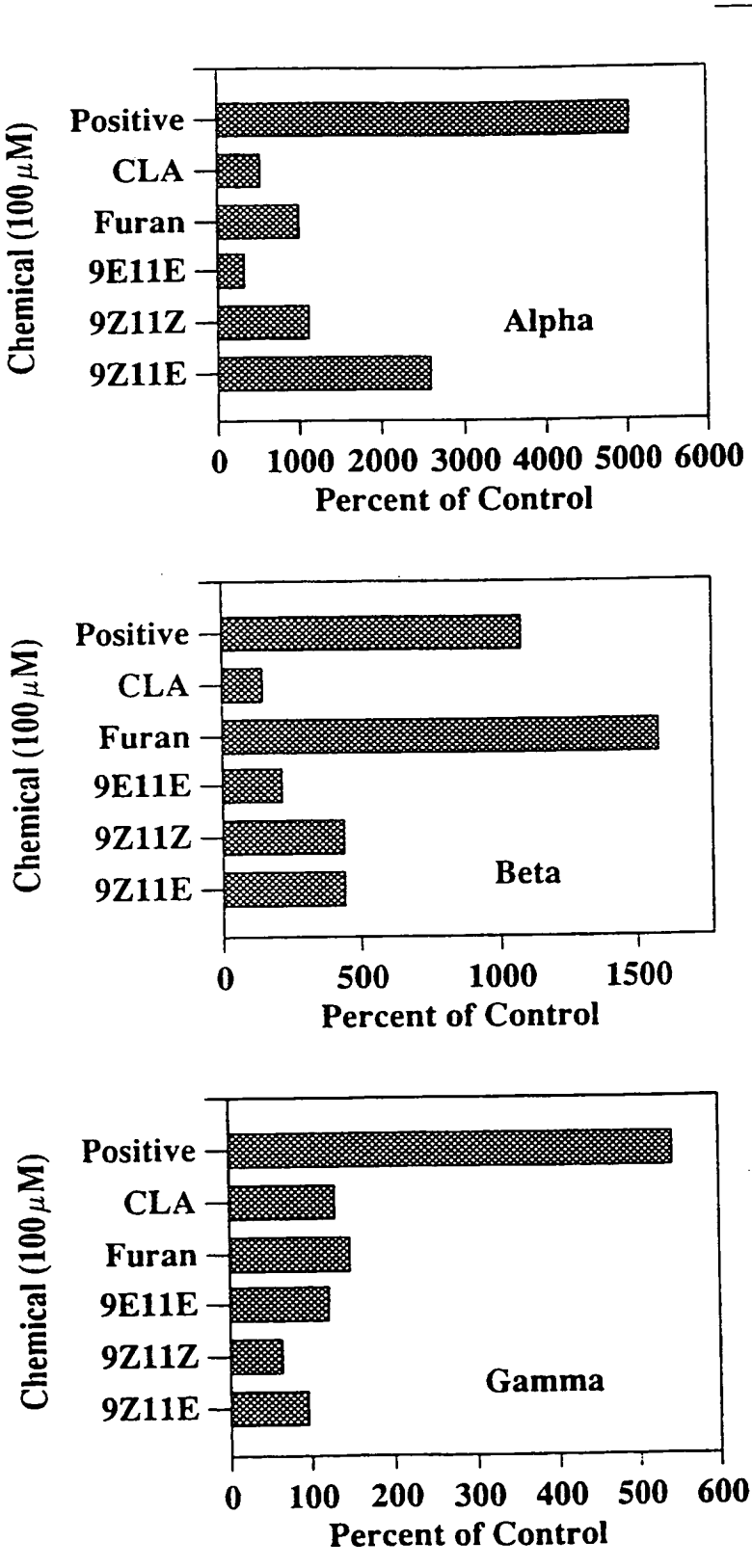
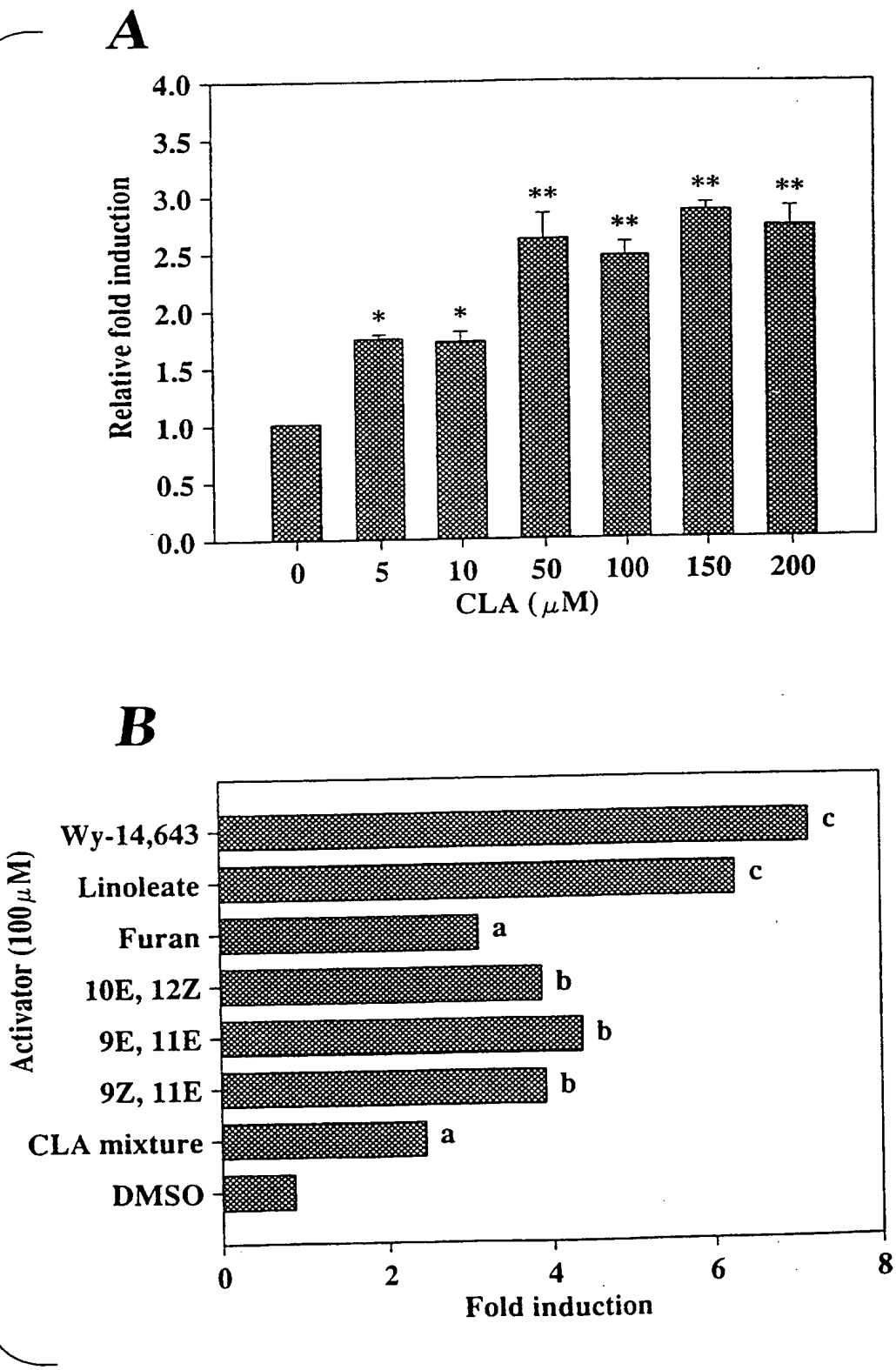

Chemical (100 μM)
Positive
CLA
Furan
9E11E
9Z11Z
9Z11E
Alpha
0 1000 2000 3000 4000 5000 6000
Percent of Control
Chemical (100 μM)
Positive
CLA
Furan
9E11E
9Z11Z
9Z11E
Beta
0 500 1000 1500
Percent of Control
Chemical (100 μM)
Positive
CLA
Furan
9E11E
9Z11Z
9Z11E
Gamma
0 100 200 300 400 500 600
Percent of Control

Fig. 4

Fig. 5


A
Relative fold induction
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
**
**
**
**
0
5
10
50
100
150
200
CLA (μM)
B
Activator (100μM)
Wy-14,643
Linoleate
Furan
10E, 12Z
9E, 11E
9Z, 11E
CLA mixture
DMSO
c
c
a
b
b
b
a
0
2
4
6
8
Fold induction

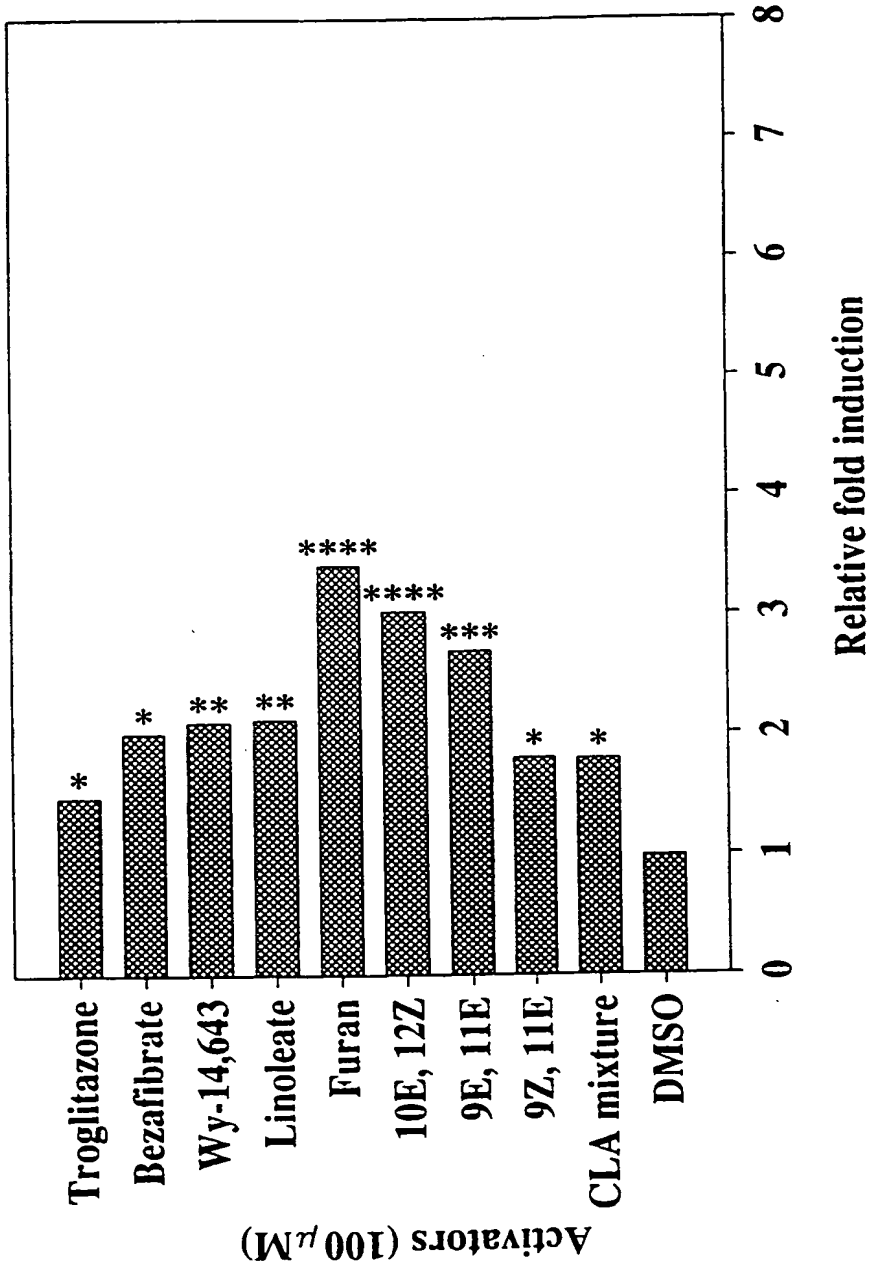

Relative fold induction
0
1
2
3
4
5
6
7
8
Activators (100 μM)
Troglitazone
Bezafibrate
Wy-14,643
Linoleate
Furan
10E, 12Z
9E, 11E
9Z, 11E
CLA mixture
DMSO
*
*
**
**
****
****
***
*
*

Fig. 6

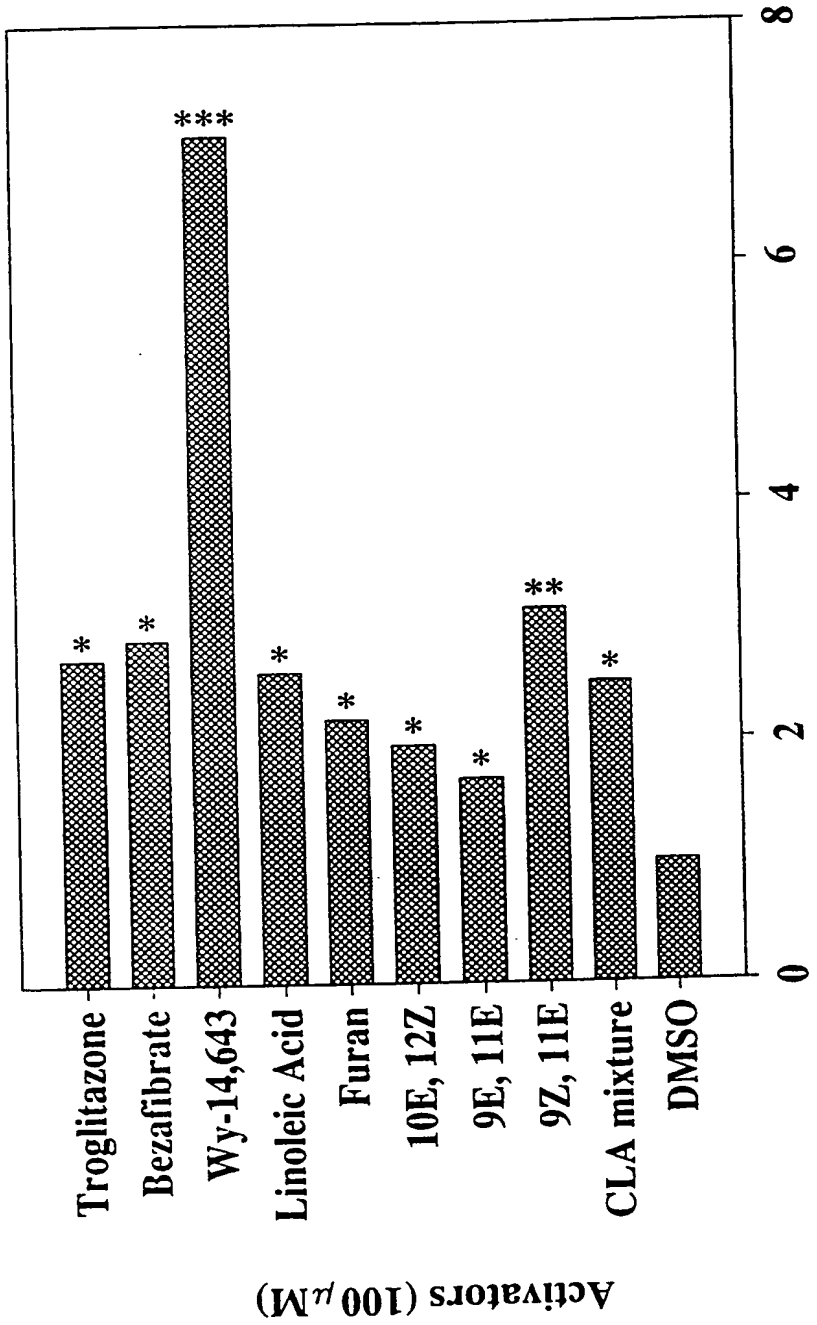

Activators (100 μM)
0
2
4
6
8
Troglitazone
Bezafibrate
Wy-14,643
Linoleic Acid
Furan
10E, 12Z
9E, 11E
9Z, 11E
CLA mixture
DMSO
*
*
***
*
*
*
*
**
*

Fig. 7

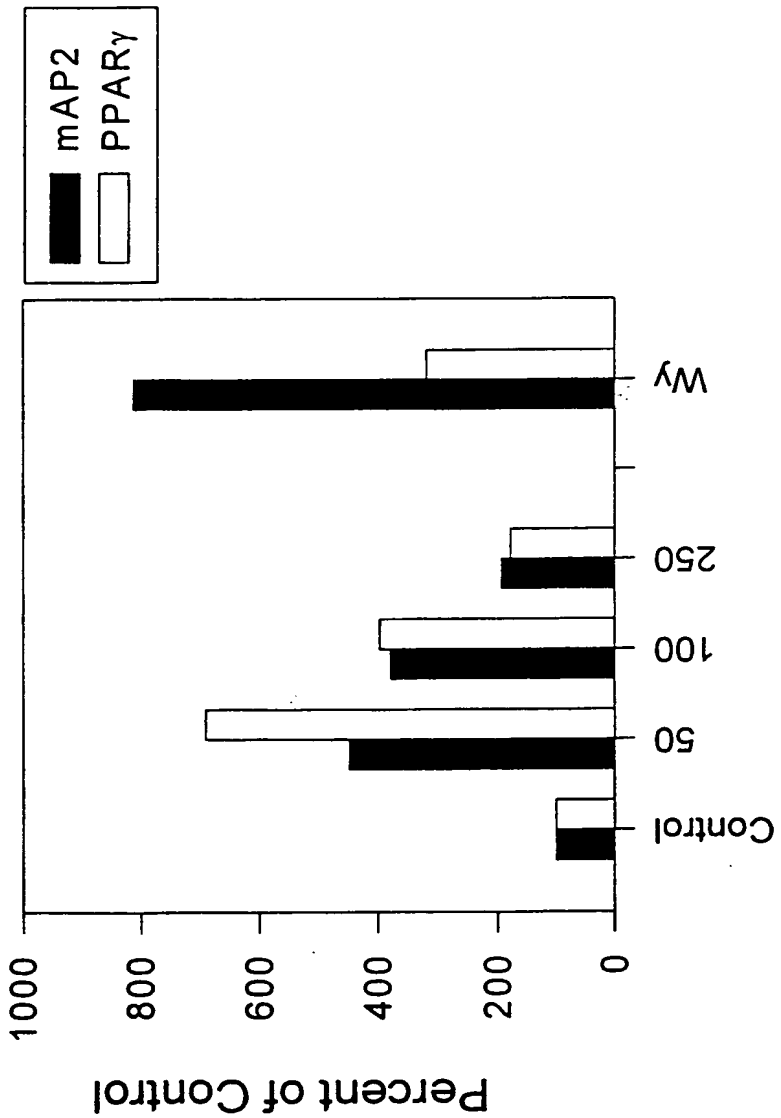

mAP2
PPARγ
Percent of Control
0
200
400
600
800
1000
Control
50
100
250
Wy

Fig. 8

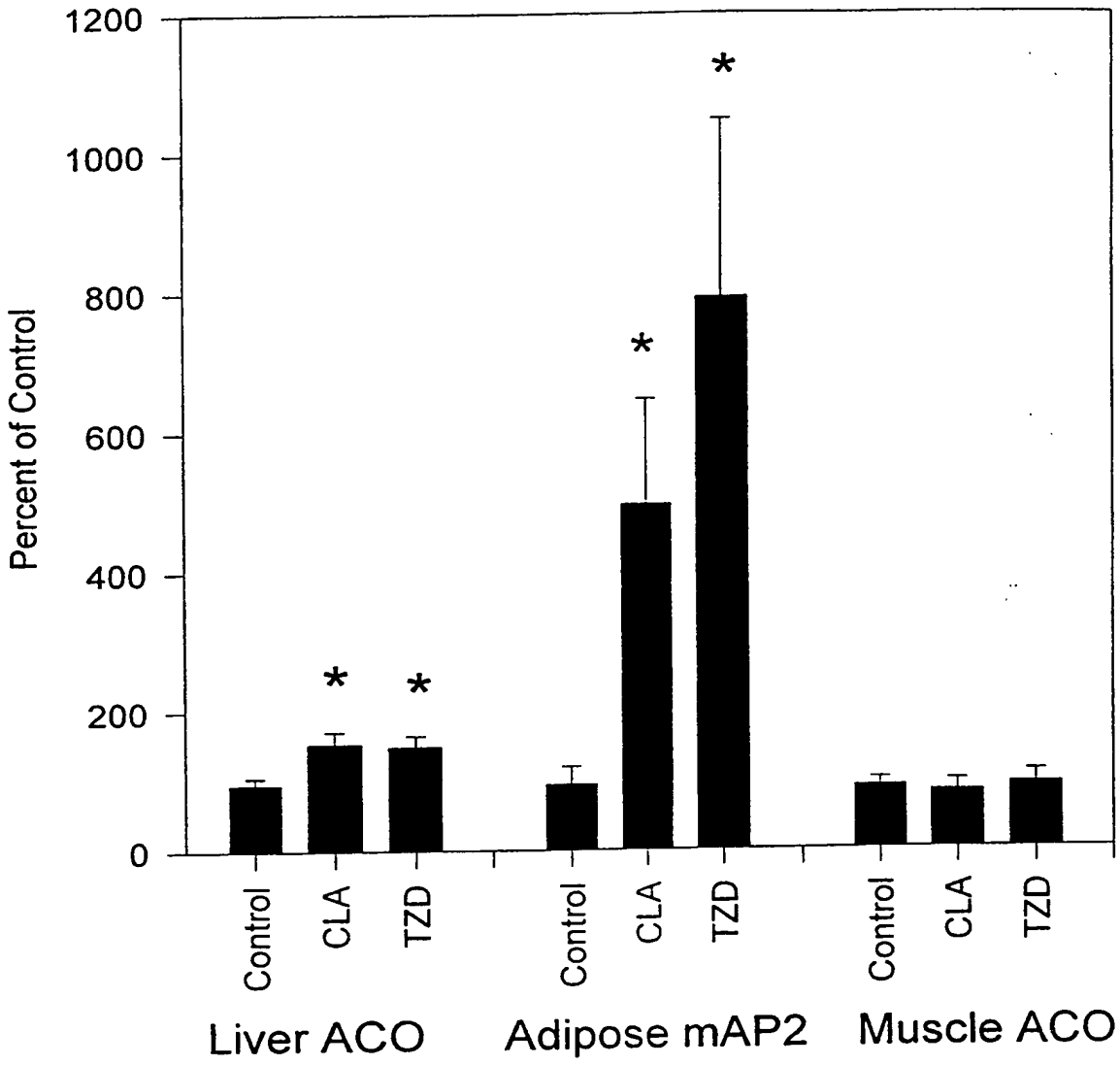

1200
1000
800
600
400
200
0
Percent of Control
*
*
*
Control
CLA
TZD
Control
CLA
TZD
Control
CLA
TZD
Liver ACO
Adipose mAP2
Muscle ACO

Fig. 9

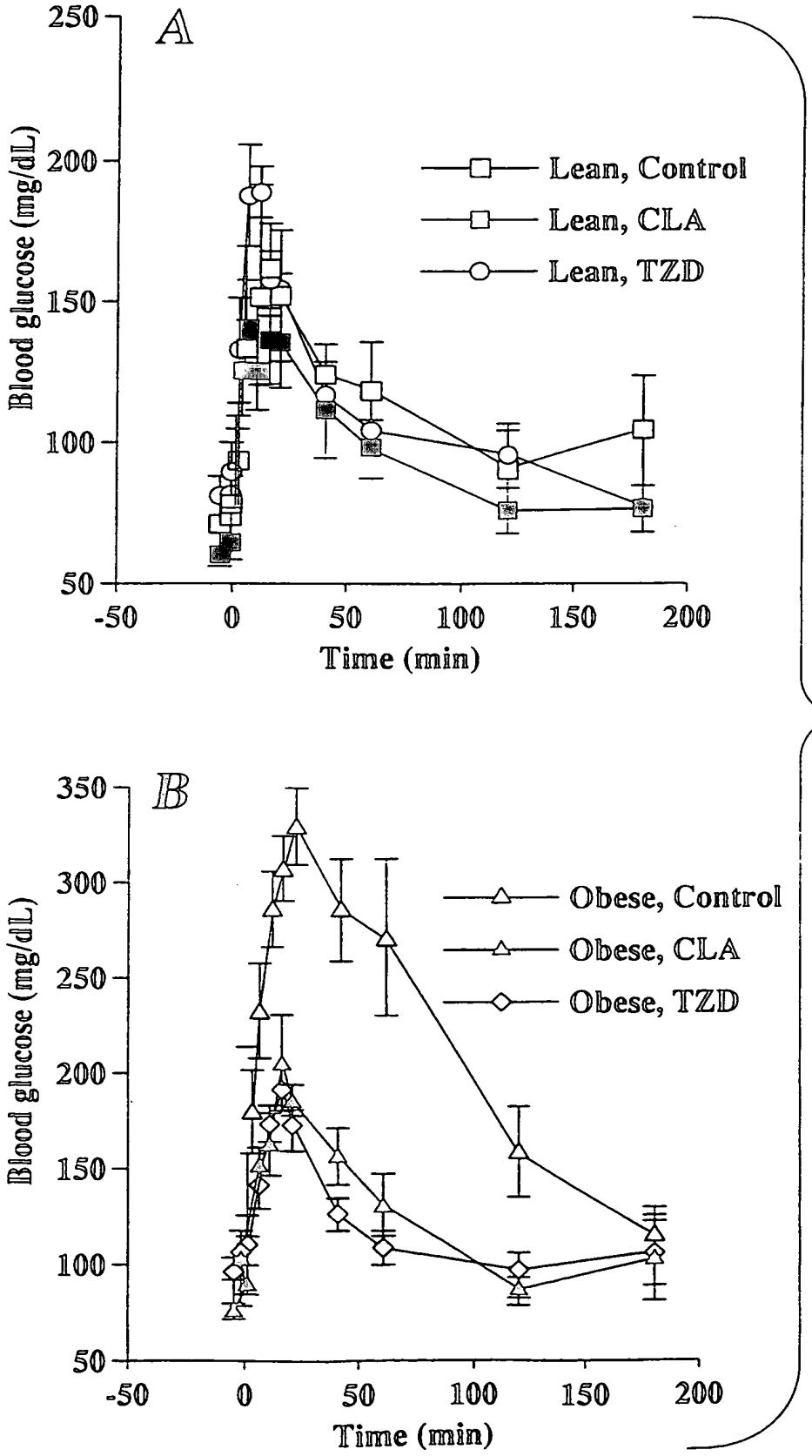

A
Blood glucose (mg/dL)
250
200
150
100
50
-50
0
50
100
150
200
Time (min)
Lean, Control
Lean, CLA
Lean, TZD
B
Blood glucose (mg/dL)
350
300
250
200
150
100
50
-50
0
50
100
150
200
Time (min)
Obese, Control
Obese, CLA
Obese, TZD

Fig. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/26469

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 31/22, 31/225
US CL :514/546, 547
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/546, 547

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: linoleic and diabetes

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,407,821 A (MENDY) 04 October 1983, column 1, line 6 to column 6, line 58.	1-21
Y	US 4,871,768 A (BISTRIAN et al.) 03 October 1989, column Column 3, line 64 to column 4, line 40.	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 23 MARCH 1999	Date of mailing of the international search report 12 APR 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer THEODORE J. CRIARES Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*